AD_____

Award Number: DAMD17-99-1-9196

TITLE: Dietary Prevention of Breast Cancer

PRINCIPAL INVESTIGATOR: Leena A. Hilakivi-Clarke, Ph.D.

CONTRACTING ORGANIZATION: Georgetown University Medical Center
Washington, DC 20057

REPORT DATE: September 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Final (15 Aug 99 - 14 Aug 02)

4. TITLE AND SUBTITLE
Dietary Prevention of Breast Cancer

5. FUNDING NUMBERS
DAMD17-99-1-9196

6. AUTHOR(S) :
Leena A. Hilakivi-Clarke, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Georgetown University Medical Center
Washington, DC 20057

E-Mail: Clarkel@georgetown.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
The goal of this Academic Award is to appraise critically the state of dietary prevention of breast cancer and to forge new avenues of investigation in the field of nutrition. This goal consists of two specific aims: (1) to determine the importance of timing of specific dietary exposures on breast cancer risk; and (2) to develop a novel nutrition and cancer program to the PI's institution. Following achievements have been made towards the Aim 1: the PI's laboratory is currently focusing on investigating the impact of an exposure, either during *in utero* period through a pregnant dam, during prepubertal period or during pregnancy, to different types of dietary fats, phytoestrogens and alcohol on breast cancer risk. The studies show that maternal exposure to some dietary components that increase pregnancy estrogen levels or activate the estrogen receptor, including n-6 polyunsaturated fatty acids (PUFAs), genistein or alcohol increase dams and/or female offspring's mammary tumorigenesis, while maternal dietary exposure to soy or n-3 PUFAs either has no effect or reduces dams and offspring's risk of developing breast cancer. Prepubertal exposure to estrogenic dietary components genistein and n-3 PUFAs reduce later breast cancer risk. We have confirmed some of the results in human studies. Importantly, excessive weight gain during pregnancy increases mother's later breast cancer risk. Aim 2: the PI has developed a course entitled "Life style and cancer prevention" to the Tumor Biology graduate program at Georgetown University. In addition, the PI has obtained a planning grant from NCI to develop a U54 program to study the interactions among nutrition, genes and cancer.

14. SUBJECT TERMS	15. NUMBER OF PAGES
dietary prevention of breast cancer, fatty acids, phytoestrogens, alcohol	15
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION:

The purpose of Academic Award was to allow me to appraise critically the state of **dietary prevention of breast cancer** and to forge new avenues of investigation in the field of nutrition. These new avenues were achieved through studies that examined the role of diet during periods of rapid mammary cell proliferation, such as fetal life, puberty and pregnancy, in influencing breast development and breast cancer risk. In addition, studies were done in human populations to investigate whether the data obtained using animal models apply to women. Dietary factors that were the focus of these studies were **polyunsaturated fatty acids, phytoestrogens, and alcohol**. Special emphasis was given to identify their mechanisms of action. In particular, the role of the two estrogen receptor isotypes (ER-α and ER-β), tumor suppressor genes BRCA1 and p53, and eicosanoid pathways (cyclooxygenase 2, COX-2) in mediating the effects of PUFA, phytoestrogens and alcohol, were assessed. These studies are also on-going. In women, intermediate biomarkers of breast cancer risk will be studied in the nipple aspirate fluid (NAF). During the funding period, I developed a course to address critical nutritional issues in breast cancer as a new initiative to the existing Tumor Biology graduate program at the Georgetown University, Department of Oncology. Finally, I received a planning grant from NCI to put together a program project proposal (U54) to investigate interactions among diet, genes and cancer. The U54 proposal was submitted in July 2002, and is currently under review.

BODY:

Task-1. Effect of dietary manipulations during sensitive developmental periods on breast cancer risk using animal models (months 1-24).

These dietary manipulations occurred during pregnancy and before puberty onset and they include:

1.1. n-3 and n-6 PUFA
1.2. Phytoestrogens
1.3. Alcohol

Research accomplished associated with Task-1

Several studies relating to Task-1 have been completed or are on-going, and the results have either been published, in press, submitted or in preparation. The papers relating to these studies are the following:

1) HILAKIVI-CLARKE L, CHO E, DEASSIS S, OLIVO S, EALLEY E, BOUKER KB, WELCH J, KHAN G, CLARKE R, CABANES A. Maternal and prepubertal diet, mammary development, and breast cancer risk. *J Nutr* 131:154S-157S, 2001.
2) CLARKE R, HILAKIVI-CLARKE LA, TROCK B. Dietary and environmental sources of estrogenicity and breast cancer risk. *Biologist* 48:216, 2001.
3) STEVENS R, HILAKIVI-CLARKE L. Hypothesis: alcohol exposure *in utero* and later breast cancer risk. *Alcohol and Alcoholism* 36:2767, 2001.

4) HILAKIVI-CLARKE LA, FORSEN T, LUOTO R,ERIKSSON J, OSMOND C, BARKER D. Tallness and overweight during childhood have opposing effects on breast cancer risk. *Br. J Cancer* 85:1680-1684, 2001
5) HILAKIVI-CLARKE L, CABANES A, OLIVO S, KERR L, BOUKER KB, CLARKE R. Do estrogens always increase breast cancer risk? *J Ster Biochem* 80:163-174, 2002.
6) JOHNSON M, KENNEY N, HILAKIVI-CLARKE L,, NEWBOLD R, CLARKE R, SHOLLER PF, LIRIO A, FOSS C, TROCK B, PAIK S, STOICA A, MARTIN MB. Cadmium mimics the effects of estrogen in vivo in the uterus and mammary gland. *Nature Med*, revision submitted 2002.
7) BENZ CC, HILAKIVI_CLARKE L, CONZEN S, DORN RV, FLEMING GF, GRANT K, GREENE G, HELLMAN S, HENDERSONC, HOOVER R, HRYNIUK W, JEFFREY S, LIPPMAN M, LUNG J, MITCHELL M, PIKE M. Expedition inspiration consensus. *Breast Cancer Res Treat* 70:213-219, 2001 (In appendix A).
8) CABANES A, OLIVO S, DE ASSIS S, GUSTAFSSON J-A, HILAKIVI-CLARKE L Prepubertal estradiol exposure increases estrogen receptor beta levels in the mammary gland and reduces 7,12-dimethylbenz[a]-antracene-induced mammary tumorigenesis in rats. Submitted to *Br J Cancer.*
9) BLOCK KI, CONSTANTINOU A, HILAKIVI-CLARKE L, HUGHES C, TRIPATHY D, TICE JA. Point-Counterpoint: Soy intake for breast cancer patients. *Integrative Cancer Therapies* 1:90-100, 2002 (In appendix A).
10) CLARKE R, LIU MC, BOUKER KB, GU Z, LEE RY, ZHU Y, SKAAR TC, GOMEZ B, O'BRIEN K, WANG Y, HILAKIVI-CLARKE L. Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling. *Oncogene*, in press 2002
11) TROCK B, WHITE L, HILAKIVI-CLARKE L, CLARKE R. Meta-analysis of soy intake and breast cancer risk. A manuscript in preparation, 2002.
12) HILAKIVI-CLARKE L, CHO E, CABANES A, DEASSIS S, OLIVO S, HELFERICH W, LIPPMAN ME, CLARKE R. Modulation of pregnancy estrogen levels by maternal dietary exposures and breast cancer risk among female rat offspring. *Clin Cancer Research* 8:3601-3610, 2002.
13) HILAKIVI-CLARKE L, CABANES A, DEASSIS S, LIAO DJ, GUSTAFSSON J-A, HELFRICH W. Soy diet during pregnancy reduces carcinogen-induced mammary tumorigenesis and causes a persistent increase in estrogen receptor beta protein levels in the rat mammary gland. A manuscript in preparation, 2002.
14) HILAKIVI-CLARKE L, CABANES A, LUOTO R, OLIVO S, DEASSIS S, CLARKE R. Pregnancy and dietary prevention of breast cancer. Experimental and Clinical Endocrinology & Diabetes, A manuscript in preparation, 2002.
15) HILAKIVI-CLARKE L, CABANES A, KHAN G , SHOEMAKER W, STEVENS R. *In utero* alcohol exposure increases mammary tumorigenesis in rats. A manuscript in preparation, 2002

In addition, studies are currently on-going to determine the impact of (1) maternal and prepubertal exposure to lignans, oats and wheats, (2) prepubertal exposure to n-3 and n-6 PUFAs, and (3) pregnancy exposure to alcohol in affecting later mammary tumorigenesis,

Summary of the findings: Elevated estrogen levels *in utero* have been shown to increase the susceptibility to breast cancer by altering breast cancer morphology and expression of estrogen-

regulated genes. In contrast, estrogen exposure before the onset of puberty reduces later breast cancer risk by narrowing the window when the breast is highly susceptible for malignant transformation. We have investigated how to modify estrogen levels in utero and during prepuberty by diet and found that maternal exposure to n-6 fatty acids, alcohol or phytoestrogen genistein during pregnancy increases female offspring's mammary tumorigenesis. However, maternal exposure to n-3 fatty acids reduces offspring's breast cancer risk, although n-3 fatty acids elevate pregnancy estrogen levels. Prepubertal exposure to genistein or n-3 fatty acids reduces later breast cancer risk. Our results suggest that timing of dietary exposures determines their effect on breast cancer risk.

Task-2. Identification of mechanistic pathways that mediate the effects of PUFA, phytoestrogens, and alcohol on breast cancer risk (months 12-36).

These intermediate biomarkers include:
2.1. ER-α and ER-β
2.2. Tumor suppressor genes BRCA1 and p53
2.3. Eicosanoids, particularly COX-2

Research accomplished associated with Task-2

Some of the findings concerning the mechanisms possibly mediating the effects of dietary manipulations on breast cancer risk are reported in the studies listed above (8, 12, 13 and 15). In addition, following published papers also address the plausible mediating mechanisms:

16) HILAKIVI-CLARKE LA. Estrogens, BRCA1 and breast cancer. *Cancer Research*, 60:4993-5001, 2000. (Submitted under 2001 report)
17) BOUKER K, HILAKIVI-CLARKE LA. Genistein: A powerful weapon in the breast cancer prevention arsenal or a potent mitogen? *Environmental Health Perspectives* 108: 701-708, 2000. (Submitted under 2001 report)

In utero alcohol exposure and breast cancer risk

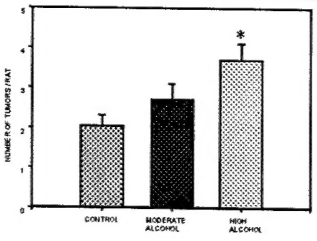

Fig 3. Tumor multiplicity (number of tumors per animal) in DMBA-treated animals exposed to moderate or high levels of alcohol *in utero* (mean ± SEM). Tumor multiplicity was significantly higher in animals exposed to high levels of alcohol *in utero*, when compared to controls (one-way ANOVA: $p<0.01$).

Studies to investigate the impact of timing of alcohol exposure on mammary tumorigenesis are currently in progress. We have completed a study investigating the effect of in utero alcohol exposure on later mammary tumorigenesis, and presented at the first annual AACR Cancer Prevention Meeting in Boston, October 14-18, 2002.

Briefly, pregnant rats were fed liquid diets containing either 16% (moderate) or 25 % (high) alcohol between gestation days 7 and 19. The control rats were pair fed with a diet containing no alcohol. The blood alcohol levels in pregnant rats fed 16% or 25% alcohol diets are comparable to those seen in women consuming moderate and high levels of alcohol, respectively. One-way ANOVA indicated that the dams exposed to moderate alcohol diet had significantly higher pregnancy E2 levels than the control dams ($p<0.05$) ($F(2,27)=7.3$, $p<0.003$).

Mammary tumors in 2-month-old female offspring of alcohol and control diet exposed dams were induced by administering 10 mg 7,12-dimethylbenz(a)anthracene (DMBA). We noted that tumor multiplicity, reflecting the total number of tumors per animal, was significantly higher in the rats exposed to alcohol *in utero*, when compared to the controls ($F(2,63)=5.88$, $p<0.005$) **(Fig. 1)**.

We further studied the mechanisms that could mediate the effects of maternal alcohol intake during pregnancy on offspring's breast cancer risk. The results indicated that mammary epithelial density was dose-dependtly increased and the glands of alcohol-exposed offspring contained more targets for malignant transformation. In addition, our findings showed that ER-α expression was increased and ER-β expression was reduced in the mammary glands of alcohol-exposed offspring. Taken together, the study provide strong evidence that in utero alcohol exposure might increase later breast cancer risk, perhaps by altering mammary gland morphology and expression of ERα and ERβ. To continue this line of research, we have submitted an RO1 grant application to NIH.

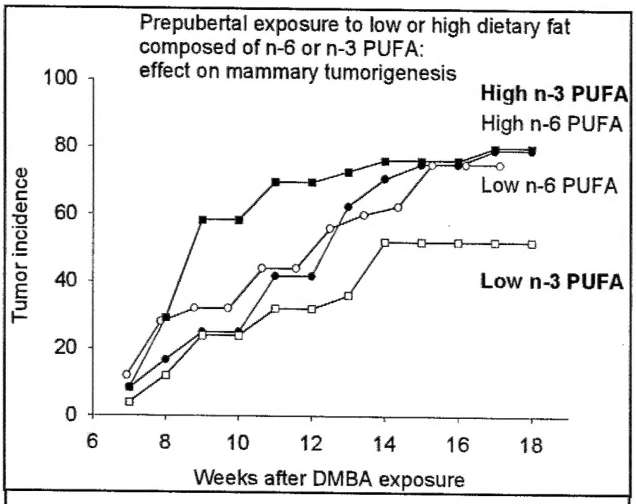

Figure 2. DMBA-induced mammary tumorigenesis in animals exposed to low or high fat diet composed of n-3 or n-6 PUFAs during prepuberty. Animals exposed to a low fat n-3 PUFA diet had the lowest mammary tumor incidence (Log Rank test: $p<0.01$).

n-3 PUFA: COX-2 pathway

We are currently investigating the effect of prepubertal exposure to n-3 or n-6 PUFAs on later mammary tumorigenesis and the pathways mediating their effects on the breast. The results indicate that prepubertal exposure to a low-fat diet containing n-3 PUFAs reduces later mammary tumorigenesis in an animal model **(Fig. 2)**. n-3 PUFAs inhibit cyclooxygenase-2 (COX-2) expression, and this enzyme is linked to increased risk of developing breast cancer, probably partly by increasing the convertion of arachidonic acid to prostaglandins. We observed that the animals fed a low or high fat n-3 PUFA diet between postnatal days 5 and 25, later on expressed significantly lower levels of COX-2 in their mammary gland than the control low fat n-6 PUFA fed animals (Kruskal-Wallis one-way ANOVA: $p<0.01$). Only 4% of the cells in the mammary glands of the animals fed a low fat n-3 PUFA diet expressed COX-2, compared to 28% and 52% in the animals fed either a low or high fat n-6 PUFA diet, respectively. In addition, the intensity of the staining was altered. The animals fed a low or high fat n-3 PUFA diet had a significantly lower COX-2 staining intensity, compared to the animals fed a low or high fat n-6 PUFA diets ($p<0.05$). These findings suggest that a feeding a low fat diet rich in n-3 fatty acids consumed during prepuberty may be an effective chemopreventive approach to inhibit breast cancer.

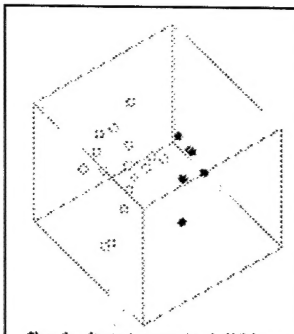

Figure 3: Structural representation of a 20-D data set reduced by the gene selection between low n-3 and n-6 and projected into a 3-D space by PCA. The top three principal components were cover 96% of the total variance spanned by the data.
Low n-3 (n=6) ●; High n-3 (n=5) ○; all n-6 (n=11) □.

TABLE 4: MLP Prediction for Protective vs. No Protective effect breast cancer risk

MLPs: Trained on Class A = Protective vs. Class B = No Protective Effect

	Training Data Sets (CLASSIFIED)		
	Protective (n=4)	No protective (n=5)	TOTAL
After 30 cycles	120/120	150/150	100%
	Evaluation Data Sets (CLASSIFIED)		
	Protective (n=2)	No protective (n=2)	TOTAL
After 30 cycles	60/60	90/90	100%
	86 ± 5%	78 ± 6%	
	Testing Data Sets (CLASSIFIED)		
	Low Susceptibility (n=0)	High Susceptibility (n=8)	TOTAL
After 30 cycles	n/a	230/248	92±1%
	n/a	92±1%	
	Low Susceptibility	High Susceptibility	
1 cycle	(n=0)	(n=8)	
Class Predicted	A:B n/a	0:8	

In addition to changes in specific pathways, we have also began to explore more global changes identified using gene microarray approach. Each gland obtained from 8-week-old animals that were fed prepubertally low or high fat diets composed of n-3 or n-6 PUFAs was arrayed independently on a pre-release version of Research Genetics Rat GeneFilters (cDNA microarrays with 4,386 rat specific cDNAs, 1,176 of which are ESTs and 3,210 of which are "known" genes). ***The data in Fig 3 and Table IV provide compelling evidence that we have identified a subset of genes that may predict the cancer susceptibility of mammary glands exposed to different PUFA diets.*** Preliminary analysis identified 20 genes that were differentially expressed among the prepubertal dietary exposures to low or high fat diets composed of n-3 or n-6 PUFAs and these genes are currently being confirmed.

Task-3. Study whether (1) dietary fat intake and weight gain alter pregnancy estrogen levels in women, (2) affect possible intermediate biomarkers of increased breast cancer risk, as determined in nipple aspirate fluid, and (3) increase subsequent breast cancer risk (months 1-36)

3.1. The interactions among diet, weight gain and circulating estrogens and growth factor levels in pregnant women.
3.2. The effects of diet and weight gain on intermediate biomarkers in NAF.
3.3. Cohort studies to investigate whether pregnancy weight gain increases subsequent breast cancer risk.

Research accomplished associated with Task-3

Findings related to 3.1 and 3.2. The PI obtained an RO1 grant from the National Cancer Institute (funding begin 9/1/01) to study associations between diet and serum hormone levels during pregnancy, and also explore the role of polymorphism in estrogen regulated genes in interacting with diet and pregnancy serum hormones. Further, we will include women to the study who are at high familial risk of developing breast cancer. For example, pregnancy always (regardless of age at first birth) increases breast cancer risk in BRCA1 mutation carriers. It is not known whether these women are particularly vulnerable to high pregnancy estrogen levels and whether the risk might not be elevated if estrogen levels remain at a lower range.

At present time we have recruited approximately 220 Swedish women to this study, and obtained their blood samples on gestation weeks 12, 25 and 32. These women have also completed three 24 hr dietary records on the same three gestation weeks. In addition, we have begun to collect nipple aspirate fluid samples that are obtained 12 months after pregnancy. Approximately 30 samples have been collected thus far. Biological assays to determine serum and nipple aspirate fluid estrogen and growth factor levels will be performed in collaboration with Dr. Stephen Soldin at the Georgetown University.

Findings related to 3.3.

We have completed two studies to determine whether pregnancy weight gain affects mothers' breast cancer risk. The findings indicate that excessive pregnancy weight gain and postpartum weight retention increases postmenopausal breast cancer, but does not appear to influence premenopausal

breast cancer risk. We have submitted the following manuscript for publication:

18) KINNUNEN TI, LUOTO R, HEMMINKI E, GISSLER M, HILAKIVI-CLARKE L. Pregnancy weight gain and breast cancer risk. Submitted 2002.
19) HILAKIVI-CLARKE L, LUOTO R, HUTTUNEN T, KOSKENVUO M. Pregnancy weight gain and breast cancer risk - a case-control study. Submitted 2002.

Task-4. Development of Course in Nutrition and Breast Cancer (months 12-18)

4.1. Outline of the course
4.2 Integration of the course to the existing Tumor Biology Course

Research accomplished associated with Task-4.

(A) A course developed by the PI and entitled "**Life-style and Cancer Prevention**" is now part of the Tumor Biology Graduate Program at Georgetown University.

(B) The PI was awarded by the National Cancer Institute a PO-type planning grant to develop a program project to study interactions between diet, genes and cancer. The title of our funded proposal was "Timing of dietary exposures and breast cancer risk: role of steroid receptors and tumor suppressor genes". The PI submitted a U54 program project application to NCI last July.

KEY RESEARCH ACCOMPLISHMENTS

Bulleted list of key research accomplishments:

- Dietary modulations occurring **during pregnancy** can alter both mother's and her female offspring's breast cancer risk.

Animal data
Dam (mother)
Dietary factors which increase dam's breast cancer risk:
high fat n-6 polyunsaturated fatty acids

Dietary factors which reduce dam's breast cancer risk:
genistein in soy isolate feed

Offspring
Dietary factors which increase offspring's breast cancer risk:
- high fat n-6 polyunsaturated fatty acids
- genistein (administered subcutaneously)
- alcohol

Dietary factors which do not affect offspring's breast cancer risk:
- genistein in soy isolate feed

Dietary factors which reduce offspring's breast cancer risk:

- high fat n-3 polyunsaturated fatty acids.

Human data

Mother

Dietary factors that increase mother's breast cancer risk:

- excessive pregnancy weight gain

➢ ***Mechanisms*** possibly mediating the effects of *in utero* dietary modifications:

- increased pregnancy estradiol levels
- increased number of cellular targets for malignant transformation
- increased expression of ER-alpha protein and reduced expression of ER-beta protein.

- Dietary modulations **before puberty** may alter later breast cancer risk.

Animal data:

- prepubertal genistein or estradiol exposure reduces the risk.
- prepubertal low-fat n-3 PUFA diet reduces the risk.

Human data:

- high body mass during childhood is associated with reduced breast cancer risk.

➢ ***Mechanisms*** mediating the effects of prepubertal dietary modifications:

- increased estrogen exposure
- reduced number of cellular targets for malignant transformation via elimination of undifferentiated epithelial structures
- reduced expression of ER-alpha protein and increased expression of ER-beta protein.
- increased expression of BRCA1

- **Translational studies in human populations** have been initiated to determine whether findings obtained in animal studies are true also for women.

Karolinska study: Interactions among pregnancy diet, estrogen levels and intermediate biomarkers of increased breast cancer risk determined in nipple aspirate fluid. - ongoing

Finnish study 1: Pregnancy weight gain and mother's later breast cancer risk.

- Excessive pregnancy weight gain increases postmenopausal, but not premenopausal breast cancer risk.

Finnish study 2: Effect of body weight at birth, during childhood and pregnancy on

the penetrance of breast cancer in women at high familial breast cancer risk. – funding being applied.

- Course addressing the role of life-style, including diet, in affecting cancer risk added to the curriculum of Tumor Biology Graduate program.
- Funding (planning grant) obtained from the National Cancer Institute to set up a program project application that focuses on nutrition, genes and cancer.

  U54 program project grant application submitted.

REPORTABLE OUTCOMES (7/1/99-9/14/02):

Manuscripts

HILAKIVI-CLARKE LA, ONOJAFE I, RAYGADA M, CLARKE R. Maternal genistein exposure during pregnancy increases breast cancer risk among female offspring. *Oncology Reports* 6:1089-1095, 1999.

HILAKIVI-CLARKE LA, ONOJAFE I, RAYGADA M, CHO E, RUSSO I, CLARKE R. Prepubertal exposure to zearalenone or genistein reduces subsequent mammary tumorigenesis. *Br. J Cancer* 80:1682-1688, 1999.

CLARKE R, SKAAR T, EL-ASHRY D, LEONESSA F, HILAKIVI-CLARKE LA. Use of ERE and reported gene constructs to assess putative estrogenic activity. *J Medical Food* 2:127-133, 1999.

HILAKIVI-CLARKE LA, CHO E, ONOJAFE I, LIAO DJ, CLARKE R. *In utero* exposure to tamoxifen increases DMBA-induced mammary tumorigenesis. *Clinical Cancer Research* 6:305-308, 2000.

HILAKIVI-CLARKE LA. Estrogens, BRCA1 and breast cancer. *Cancer Research*, 60:4993-5001, 2000.

CABANES A, DE ASSIS S, GUSTAFSSON J-A, HILAKIVI-CLARKE L, . Maternal high-fat intake during pregnancy increases voluntary alcohol intake and hypothalamic estrogen receptor protein levels among female offspring. *Dev Neuroscience*, 22:488-93, 2000.

BOUKER K, HILAKIVI-CLARKE LA. Genistein: A powerful weapon in the breast cancer prevention arsenal or a potent mitogen? *Environmental Health Perspectives* 108: 701-708, 2000.

HILAKIVI-CLARKE L, CHO E, DEASSIS S, OLIVO S, EALLEY E, BOUKER KB, WELCH J, KHAN G, CLARKE R, CABANES A. Maternal and prepubertal diet, mammary development, and breast cancer risk. *J Nutr* 131:154S-157S, 2001.

CLARKE R, HILAKIVI-CLARKE LA, TROCK B. Dietary and environmental sources of estrogenicity and breast cancer risk. *Biologist* 48:21-6, 2001.

STEVENS R, HILAKIVI-CLARKE L. Hypothesis: alcohol exposure *in utero* and later breast cancer risk. *Alcohol and Alcoholism* 36:276-7, 2001.

HILAKIVI-CLARKE LA, FORSEN T, LUOTO R,ERIKSSON J, OSMOND C, BARKER D. Tallness and overweight during childhood have opposing effects on breast cancer risk. *Br J Cancer*, 85:1680-1684, 2001.

HILAKIVI-CLARKE L, CABANES A, OLIVO S, KERR L, BOUKER KB, CLARKE R. Do estrogens always increase breast cancer risk. *J Steroid Biochem* 80:163-174, 2002.

BENZ CC, HILAKIVI-CLARKE L, CONZEN S, DORN RV, FLEMING GF, GRANT K, GREENE G, HELLMAN S, HENDERSON C, HOOVER R, HRYNIUK W, JEFFREY S, LIPPMAN M, LUNG J, MITCHELL M, PIKE M. Expedition inspiration consensus. *Breast*

Cancer Res Treat 70:213-219, 2001.

BLOCK KI, CONSTANTINOU A, HILAKIVI-CLARKE L, HUGHES C, TRIPATHY D, TICE JA. Point-Counterpoint: Soy intake for breast cancer patients. Integrative Cancer Therapies 1:90-100, 2002.

HILAKIVI-CLARKE L, CHO E, CABANES A, DEASSIS S, OLIVO S, HELFERICH W, LIPPMAN ME, CLARKE R. Modulation of pregnancy estrogen levels by maternal dietary exposures and breast cancer risk among female rat offspring. *Clin Cancer Research*, in press, 2002

CLARKE R, LIU MC, BOUKER KB, GU Z, LEE RY, ZHU Y, SKAAR TC, GOMEZ B, O'BRIEN K, WANG Y, HILAKIVI-CLARKE L. Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling. *Oncogene*, in press 2002

JOHNSON M, KENNEY N, HILAKIVI-CLARKE L,, NEWBOLD R, CLARKE R, SHOLLER PF, LIRIO A, FOSS C, TROCK B, PAIK S, STOICA A, MARTIN MB. Cadmium mimics the effects of estrogen in vivo in the uterus and mammary gland. *Nature Med*, revision submitted 2001.

CABANES A, OLIVO S, DE ASSIS S, GUSTAFSSON J-A, HILAKIVI-CLARKE L Prepubertal estradiol exposure increases estrogen receptor beta levels in the mammary gland and reduces 7,12-dimethylbenz[a]-antracene-induced mammary tumorigenesis in rats. Submitted, 2001.

Manuscripts in preparation

TROCK B, WHITE L, HILAKIVI-CLARKE L, CLARKE R. Meta-analysis of soy intake and breast cancer risk. A manuscript in preparation, 2002.

HILAKIVI-CLARKE L, CABANES A, DEASSIS S, GUSTAFSSON J-A, HELFERICH W. Soy diet during pregnancy reduces carcinogen-induced mammary tumorigenesis and causes a persistent increase in estrogen receptor β protein levels in the rat mammary gland. A manuscript in preparation, 2002.

HILAKIVI-CLARKE L, CABANES A, LUOTO R, OLIVO S, DEASSIS S, CLARKE R. Pregnancy and dietary prevention of breast cancer. Experimental and Clinical Endocrinology & Diabetes, under preparation 2002.

HILAKIVI-CLARKE L, CABANES A, KHAN G , SHOEMAKER W, STEVENS R. *In utero* alcohol exposure increases mammary tumorigenesis in rats. A manuscript in preparation, 2002

Presentations

1. Annual Conference of American Institute for Cancer Research. A symposium presentation in the session "Nutrition, Normal Development and Cancer Prevention" entitled "*Maternal and prepubertal diet, mammary development and breast cancer risk*". In Washington DC, September 2000.

2. The Susan G. Komen *Reaching for the Cure ... Making A Difference* Mission Conference. Oral presentation entitled "*Timing of estrogen exposure and breast cancer risk*". In Washington DC, September 2000.

3. The Third Cooper Institute Scientific Conference: Physical Activity and Cancer. A symposium presentation entitled "*Physical activity, stress adaptation - habituation, and cancer*". In Dallas, Texas. November 5-7, 2000.

4. 5th Annual Breast Cancer Symposium by the Expedition Inspiration Fund for Breast Cancer Research, entitled "Hormones and Breast Cancer: Pathogenesis, Prevention, Treatment." March,

2000.

5. Annual Oncology Conference, University of Missouri. Keynote presentation, entitled *"Timing of genistein exposure and breast cancer risk."* Missouri, April 27, 2000.

6. Gordon Conference entitled "Hormonal carcinogenesis", presentation in the late breaking research session entitled *"Dietary factors in hormonal carcinogenesis."* Kimball Union Academy, July 8-13, 2001.

7. NCI Workshop on Gene-Environment Interactions in the Etiology of Childhood Cancer. A presentation entitled *"Effect of early life Dietary Exposures on Mammary carcinogenesis"*. Bethesda, Maryland, 2002.

8. American Association for Cancer Research. Presentation *"Timing of estrogen exposure and breast cancer risk"* at an educational session #12 "Gene expression and pathways to cancer and cancer risk". San Francisco, April 2002.

Funding

ACTIVE SUPPORT

Susan G. Komen Breast Cancer Foundation (PI: Hilakivi-Clarke)
10% 03/1/99-12/31/02
No current funding (Grant is in a no-cost extension period.)
"Pregnancy estrogens, diet, and intermediate biomarkers of breast cancer risk in nipple aspirate fluid"
This grant have provided seed funding to investigate the role of diet in affecting pregnancy estrogens, and their possible connection to biomarkers of increased breast cancer risk. In addition, the funds have been used to investigate whether pregnancy weight gain affects breast cancer risk in two cohorts, in collaboration with Dr. Riitta Luoto, University of Tampere, Finland.

US Army Medical Research and Material Command (PI: Hilakivi-Clarke)
DAMD17-99-1-9196 08/15/99-09/14/02 100% for salary support*
$141,638
"Dietary prevention of breast cancer"
This is a career development award that provided salary support for the PI to critically appraise the state of dietary prevention of breast cancer.

NIH/NCI (PI: Hilakivi-Clarke)
5 RO1 CA89950-02 08/15/01-7/31/06 30%
$200,000
"Pregnancy estrogens, diet, and breast cancer risk"
This grant will provide funding to a project to be conducted in collaboration with Karolinska Institute in Sweden to study factors during pregnancy that might influence breast cancer risk in mothers.

Cancer Research Foundation of America (PI: Westerlind, Kim)
09/15/01-09/15/02 5%
$35,000
"Prepubertal physical activity and rat mammary tumorigenesis"
This project will study whether prepubertal physical activity affects mammary tumorigenesis and the mechanisms mediating the association.

NIH/NCI (PI: Hilakivi-Clarke)
1 P20 CA93986 09/18/01-03/18/03 5%
No current funding (Grant is in a no-cost extension period.)
"Timing of dietary exposures and breast cancer risk: role of steroid receptors and tumor suppressor genes"
Planning Grant to establish collaborations on nutritional modulation of genetic pathways leading to cancer and apply for U54 grant.

Susan G. Komen Breast Cancer Foundation (PI: Hilakivi-Clarke) 30%
BCTR0100741 10/01/01-09/30/03
$125,520
"Timing of estrogen exposure and breast cancer risk"
This grant will provide funding to investigate the biological mediators of *in utero* and prepubertal estrogenicity on breast cancer risk.

Breast Cancer Research Foundation (PI: Hilakivi-Clarke) 10%
BCRA-01 10/01/01-09/30/03
$250,000
"Early life exposure to whole grains and breast cancer risk"
This project investigates whether some breast cancers can be prevented by dietary exposure to fiber, either during fetal life through pregnant mother or during childhood.

American Institute for Cancer Research (PI: Hilakivi-Clarke) 20%
$135,000 01/01/03-01/01/05
"Prepubertal soy diet and breast cancer risk: role of estrogen receptors and tumor suppressor"
This project will investigate whether prepubertal feeding of soy reduces mammary tumorigenesis and the pathways involved mediating the effect.

PENDING SUPPORT
NIH/NCI (PI: Hilakivi-Clarke) 04/01/03-03/31/08 50%
U54 $1,269,822
"Timing of dietary exposures and breast cancer risk"
The purpose is to study the effect of timing of dietary exposures in modulating genetic pathways leading to cancer in collaborations with several investigators at different Universities and Institutions in the USA, Finland, Sweden and France.

NIH/NCI (PI: Hilakivi-Clarke) 07/01/03-07/01/07 30%
RO1 $1,000,000
"Alcohol and breast cancer: role of ER-alpha, ER-beta and BRCA1"
The goal of this proposal is to determine whether alcohol is involved in pre-initiating, initiating or promoting breast cancer, and identify possible mediating mechanisms.

CONCLUSIONS:

Summary of the results

The data obtained during the course of the 3-year Academic Award funding emphasize that dietary estrogens have different effects on breast cancer risk, depending on the timing of exposure. We found that *in utero* exposure to some dietary components (high fat n-6 PUFA, genistein, alcohol) which either increase pregnancy estrogen levels or activate the estrogen receptor increase offsprings' mammary tumorigenesis, while some have no effect (genistein in soy isolate) and some (high fat n-3 PUFA) reduce the risk. These results suggest that soy isolate and n-3 PUFA contain components that can counteract the effect of high *in utero* estrogen levels on breast cancer risk. Studies are currently in progress in human populations to determine whether (1) diet during pregnancy affects pregnancy estrogen levels and intermediate biomarkers of elevated breast cancer risk in nipple aspirate fluid; (2) pregnancy weight gain affects later breast cancer risk; and (3) indicators of high pregnancy estrogen levels affect penetrance of familial breast cancer.

Prepubertal exposure to estrogenic exposures paradoxically reduce later breast cancer risk. Our results obtained in animal models suggest that both prepubertal exposure to estradiol, genistein or low fat n-3 PUFA diet reduces the likelihood that rats will develop carcinogen-induced mammary tumorigenesis. Data obtained in a cohort study support the protective effect of high childhood estrogenic environment and show that high body mass during childhood is linked to reduced breast cancer risk.

The results strongly suggest that a new variable, timing of exposure should also be taken into account when the effects of diet on the risk of developing breast cancer are being assessed.

Our data further indicate that the mechanisms by which early life dietary exposures affect breast cancer risk are related to changes in the mammary gland differentiation and expression of ER-α, ER-β, and BRCA1. In particular, increase in ER-β protein and BRCA1 levels might protect the breast from malignant transformation. Our ultimate goal is to identify the best means to prevent some breast cancers by dietary modifications during childhood and pregnancy.

So what section

Although diet is clearly associated with breast cancer risk, studies have failed to provide convincing evidence in favor of a particular dietary component in causing or preventing breast cancer. This failure is likely to have been caused by not having been taking into consideration the fact that timing of exposure is critical. An exposure to the same dietary component might have a different effect on breast cancer risk, if the exposure occurs *in utero* through a pregnant mother, during childhood, puberty, pregnancy, reproductive years or postmenopause. For example, our recent results and the results of other investigators indicate that an exposure to a phytoestrogen genistein during fetal life and postmenopause might increase breast cancer risk, while an exposure during childhood may provide a permanent protection. By determining the impact of timing of various dietary components, we are more likely to be able to prevent some breast cancers than by assessing the interaction between diet shortly before diagnosis and breast cancer risk.